

## REMARKS

The specification is amended to include a priority claim to the parent applications. All of the other amendments to the specification merely are directed to grammatical and other minor wording changes, and are consistent with amendments made in the parent case. Support for these amendments is present surrounding the locations where the amendments have been entered, because the nature of the amendments are clear from the context of the application.

In the present amendment, the Applicant cancels all claims 1-8, and adds new claims 9-10 to the application. Thus, upon entering these amendments, claims 9-10 will be pending in the application. Claims 9-10 were subject to restriction in the parent application and canceled at the time of allowance.

The Applicant does not intend by these claim amendments or any other amendments to abandon the subject matter of any claim as originally filed or as later presented. The Applicant reserves the right to pursue such subject matter in subsequent applications, such as continuations, CIP's, and divisionals.

The amendments include no new matter.

Respectfully submitted,

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## APPENDIX OF CHANGES IN THE SPECIFICATION

At page 1, line 1:

This application is a divisional application of U.S. patent application Serial No: 09/029,156, filed August 3, 1998, which is 35 U.S.C. §371 national filing of PCT/JP96/00173, filed January 25, 1996, which in turn is a continuation-in-part of PCT/JP95/02035, filed 02 October 1995, now U.S. Patent No. 6,110,708

At page 2, lines 19-25:

Similar methods had also been tried by using another expression vectors, but the same or less expression level had merely detected by any of the vectors. Anyway, an effective expression system have [not been realized yet] not yet been realized in the art. This seems due to difficulties in expressing the conglutinin because *Escherichia coli* does not possess proteins of the structure like collagen-like region. Further, yield of the conglutinin produced from an eukaryotic cells is little, and some of the conglutinin may sometimes have an inappropriate post-transcriptional modification.

At page 4, lines 9-10:

Figure 8 shows conglutination activities on the recombinant conglutinin and the native conglutinin with[mictotier] microtiter plate assay system;

At page 5, lines 21-25:

PCR products of Example 1(1) were digested with the restriction enzymes XhoI and EcoRI, then were inserted into the expression vector pRSET-A (Invitrogen) with DNA ligation kit (Takara Shuzo). [Ligation solution was then] Then, pRSET vector so prepared from pRSET-A containing bovine conglutinin cDNA fragment was transfected into Escherichia coli JM109 and transformants [was] were obtained that have the conglutinin DNA fragments corresponding to 631 bp through 1113 bp of the native conglutinin DNA (Figure 1).

At page 5, line 26, to page 6, line 2:

Sequences of these fragments were corresponding to 191<sup>st</sup> through 351<sup>st</sup> amino acids of native conglutinin, namely, PCR exactly amplified the sequences having the short collagen region, the neck region and the carbohydrate recognition domain. Further, there was no error in the PCR reaction. Accordingly, [desirous] desirable stable transformants were obtained which can remarkably produce such conglutinin DNA fragments.

At page 10, lines 1-9:

After coating the microtiter plates with yeast mannan (1 µg/well), the recombinant conglutinins were reacted with sugars. Sugar binding specificity (I<sub>50</sub>) was shown as sugar concentration to halve binding activities. Results are shown in Table 1. Obviously from Table 1, sugar binding activities with the recombinant conglutinin are substantially same to that of the native conglutinin. Then, as shown in Figures 6 and 7, like the native conglutinin, binding activities of the recombinant conglutinin were [depended] dependent on calcium ion. Further, these binding activities were inhibited by N-acetylglucosamine. On the other hand, tags of histidine fused to the recombinant conglutinin were not involved in the binding activities to mannan and binding specificities.

At page 11, lines 25-30:

Conglutination activities [by] of the recombinant conglutinin and the native conglutinin were evaluated by Microtiter plate assay system. Sheep erythrocyte cells with iC3b were prepared according to the method of Wakamiya *et al.*, (*Biochem. Biophys. Res. Comm.*, Vol. 187, pp. 1270-1278, 1992). Namely, 1 % sheep erythrocyte cells with iC3b were prepared by priming with a mixture of ten-fold diluted fresh horse serum and equivalent amount of anti-Forssmann antibody, and incubated at 37 °C for ten minutes.

At page 12, lines 1-10:

50 µl of 1 % sheep erythrocyte cells with iC3b and 50 µl of the recombinant conglutinin or 50 µl of the native conglutinin [was] were added to the raw veronal buffer or the veronal buffer containing 30 mM N-acetylglucosamine. Then, they were incubated at 37 °C and the conglutination activities thereon were detected. The lowest concentration of the proteins to cause agglutination is regarded as titer of conglutination, then the results are shown in Figure 8. In Figure 8, Lane A is the native conglutinin, Lane B is the recombinant conglutinin and Lane C is the recombinant conglutinin containing 30 mM N-acetylglucosamine. Titer of conglutination on the native conglutinin was 0.16 µg/ml, while that of the recombinant conglutinin was 1.3-2.5 µg/ml. Such activities were completely inhibited by 30 mM N-acetylglucosamine (GlcNAc).

At page 12, line22, to page 13, line 4:

In accordance with the method of Okuno *et al.*, (*J. Clin. Microbiol.*, Vol. 28, pp. 1308-1313, 1990), experiments were performed [by] in 96-well microtiter plates with 1 % chick's erythrocytes. The ether-treated virus antigens from [an] a hen egg antigen was used. No additive had been added to mixed cultivation solution of TBS/C (TBS solution containing 5 mM sodium chloride) except for 30 mM N-acetylglucosamine or 10 mM EDTA. After incubation at room temperature for one hour, effects on the recombinant conglutinin fragments (rBKg-CRD) against viral hemagglutination on chick's erythrocytes were observed. Results are shown in Table 2. Results on Influenza A virus A/Ibaraki/1/90 are shown in Figure 9. In Figure 9, Lane A is the native conglutinin, and Lanes B, C and D are directed to the recombinant conglutinin fragments, in which the Lane B is no additives, Lane C is added thereto 30 mM N-acetylglucosamine and Lane D is added thereto 10 mM EDTA.

At page 13, lines 18-22:

Hemagglutination Inhibition (HI) activities were depended on dosages and calcium. Further, Hemagglutination Inhibition (HI) activities of the recombinant conglutinin [is] are substantially the same level to the titer of the native conglutinin, rat surfactant protein D, human surfactant protein D (*Hartshorn et al., J. Clin. Invest.*, Vol. 94, pp. 311-319, 1994).

At page 15, lines 19-25, and substitute the following paragraph:

Physiological activities against Influenza A viruses were evaluated in accordance with the evaluation method on Hemagglutination Inhibition (HI) Activities according to Example 5, the evaluation method on Neutralization Activities according to Example 6, the evaluation on Hemagglutinin (HA) Activities by Western blotting, and the present method referred to in Example 7. Further, the neutralization [activities against] activities against Influenza A viruses by the various collectins were also evaluated by the method referred to in Example 6. Results were shown in the following Tables 4 and 5.

**APPENDIX OF CURRENTLY PENDING CLAIMS UPON ENTRY OF THIS  
AMENDMENT**

Claims:

9. A purified Mannan-binding protein (MBP) having budding inhibition activity as determined by a method comprising steps of:

- (a) culturing cells in presence of virus(es) to prepare virus(es)-infected cells,
- (b) presenting the virus(es)-infected cells in presence or absence of the MBP,
- (c) comparing gross area of virus(es)-infected focus formed in the presence of the MBP with that formed in the absence of the MBP, and
- (d) evaluating from the comparison results in the step (c) an inhibition level by the MBP on budding of virus in said infected cells.

10. A purified Human Mannan-binding protein (hMBP) having anti-Influenza A virus activity as determined by the method comprising the steps of:

- (a) culturing cells in presence of virus(es) to prepare virus(es)-infected cells,
- (b) presenting the virus(es)-infected cells in presence or absence of a calcium-dependent lectin,
- (c) comparing gross area of virus(es)-infected focus formed in the presence of a calcium-dependent lectin with that formed in the absence of a calcium-dependent lectin, and
- (d) evaluating from the comparison results in the step (c) an inhibition level by the calcium-dependent lectin on budding of virus in said infected cells,  
wherein the calcium-dependent lectin comprises an N-terminal region containing cysteine, a collagen-like region, a neck region and a carbohydrate recognition domain.